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Article

1,2-Hydroxypyridonate/Terephthalamide Complexes of Gadolinium(III): Synthesis, Stability, Relaxivity, and Water Exchange Properties[1]

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Four new Gd(III) complexes based on the 1,2-hydroxypyridinone chelator have been synthesized and evaluated as potential magentic resonance imaging contrast agents. Previously reported work examining Gd-TREN-1,2-HOPO (**3**; HOPO = hydroxypyridinone) suggests that the 1,2-HOPO unit binds strongly and selectively to Gd(III), encouraging further study of the stability and relaxivity properties of this class of compounds. Among the new complexes presented in this paper are the homopodal Gd-Ser-TREN-1,2-HOPO (Gd-**5**) and three heteropodal bis-1,2-HOPO-TAM complexes (Gd-**6**, Gd-**7**, and Gd-**8**; TAM = terephthalamide). Conditional stability constants were determined, and all pGd values are in the range of 18.5−19.7, comparable to other analogous HOPO complexes and currently used commercial contrast agents. Relaxivities for all complexes are about twice those of commercial agents, ranging from 7.8 to 10.5 mM^{-1} s^{-1} (20 MHz; 25 °C), and suggest two innersphere water molecules in fast exchange. Luminescent measurements were used to verify the number of coordinated waters for Gd-**5**, and VT ^{17}O NMR experiments were employed for the highly soluble Gd-TREN-bis-1,2-HOPO-TAM-N3 (Gd-**8**) complex to measure a fast water exchange rate, $^{298}k_{ex} = 1/\tau_M$, of 5.1 (±0.4) × 10^8 s^{-1} ($^{298}\tau_M \sim 2$ ns).

Introduction

Gadolinium(III) complexes have found extensive use as contrast media for magentic resonance imaging (MRI) over the past 20 years.[2−4] While the current commercial agents based on aminocarboxylate ligand scaffolds provide some contrast enhancement, there exists much room for improvement with regard to their proton relaxation efficiency, particularly at the higher magnetic field strengths being introduced clinically. The desire for improved agents encourages the design of new Gd(III) chelates that attempt to optimize the several parameters influencing relaxivity (increase in water proton longitudinal relaxation rate per concentration of agent) while maintaining high solubility and stability for consideration as practical agents. Previous work has described a series of complexes based on the hydroxypyridinone (HOPO) and terephthalamide (TAM) binding units that shows promise as a new family of practical, high-relaxivity agents for current and future imaging applications. These Gd(III) complexes typically possess two[5] or three[6,7] innersphere water molecules and have relaxivities that range from 8 to 15 mM^{-1} s^{-1} at clinically relevant field strengths (20−100 MHz). Importantly, the chelate stabilities of these agents are comparable with, and in some cases higher than, those of commercial agents.

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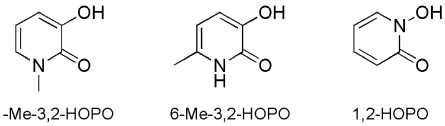

Figure 1. Three isomers of hydroxypyridinone (HOPO). Once deprotonated at the phenolic oxygen, these chelators readily bind a variety of hard metal ions.

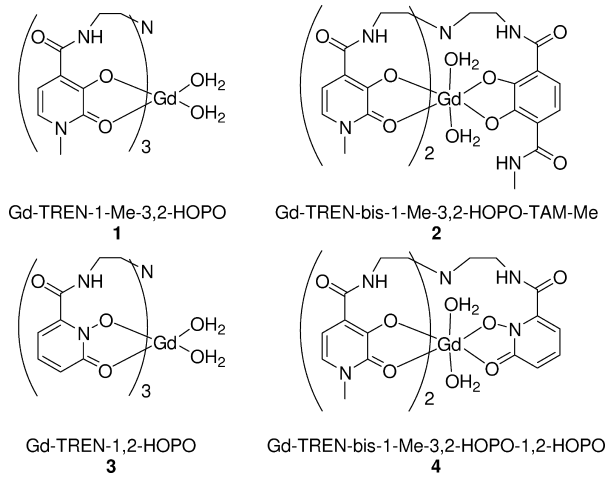

Figure 2. Chemical structures of parent TREN-3,2-HOPO Gd(III) complexes and more recently reported 1,2-HOPO derivatives.

This paper presents the development of new Gd(III) complexes based on the 1,2-hydroxypyridinone (1,2-HOPO) chelator (Figure 1). These water-soluble derivatives enable thorough investigation of the physical properties of 1,2-HOPO-based chelates as MRI contrast agents. Until recently, much of the progress in developing next-generation, high-relaxivity agents in the HOPO class of Gd(III) complexes has focused on the 3,2-HOPO chelator (Figure 1).[5,8] Used extensively in the past for applications in iron chelation[9,10] and actinide decorporation agents,[11] 3,2-HOPO is an excellent ligand for hard metal ions. Once deprotonated at the phenolic oxygen, the monoanionic HOPO chelator acts as a bidentate ligand, and when several such heterocyclic rings are attached to an appropriate scaffold, potent multidentate ligands can result.[8,12] Following the original report of Gd-TREN-1-Me-3,2-HOPO (**1**; Figure 2) as a potential contrast agent, a series of homo- and heteropodal ligands were prepared in efforts to better understand and further optimize the various parameters impacting stability and relaxivity properties.[5,13] This series primarily included 1- or 6-methyl 3,2-HOPO ligands (Figure 1) as well as mixed bis-HOPO-TAM complexes such as TREN-bis-1-Me-3,2-HOPO-TAM (**2**; Figure 2).

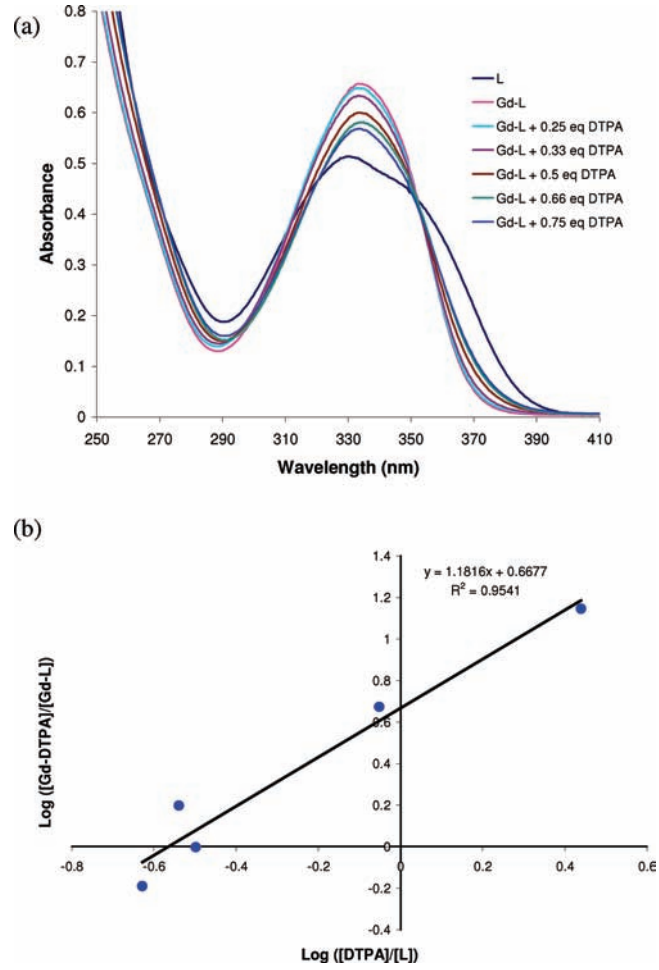

Figure 3. (a) Absorption spectra obtained from competition titration of Ser-TREN-1,2-HOPO (**5**) versus DTPA for Gd(III). Experimental conditions: [L] = [Gd] = 40 μM, [DTPA] = 10−30 μM, pH 7.4, 25 °C, 0.1 M KCl. (b) Competition titration log/log plot for **5** versus DTPA; the *x* intercept indicates the difference in pGd between **5** and DTPA.

While 3,2-HOPO chelators have been extensively featured in the development of HOPO-based contrast agents, the more acidic 1,2-HOPO isomer has received limited attention. The first literature report examining 1,2-HOPO as a Gd(III) chelator appeared in 2004 with the publication of the crystal structure of Gd-TREN-1,2-HOPO (**3**; Figure 2).[14] Relaxivity measurements were performed for **3** as well as for the TREN-bis-1-Me-3,2-HOPO-1,2-HOPO complex (**4**), and the values obtained of 9.5 and 9.3 mM^{-1} s^{-1} (20 MHz, 25 °C) are consistent with two coordinated water molecules and fast water exchange. It was also shown that at low magnetic fields, where electronic relaxation times sensibly influence relaxivity, the relaxivity values increased with an increasing number of 1,2-HOPO chelators offered by the ligand. This suggests a possible effect of the 1,2-HOPO isomer on electron spin relaxation of the metal center. A followup study was recently published which further probed the stability properties of the parent Gd-TREN-1,2-HOPO complex.[15] Such detailed stability and selectivity studies, as well as the

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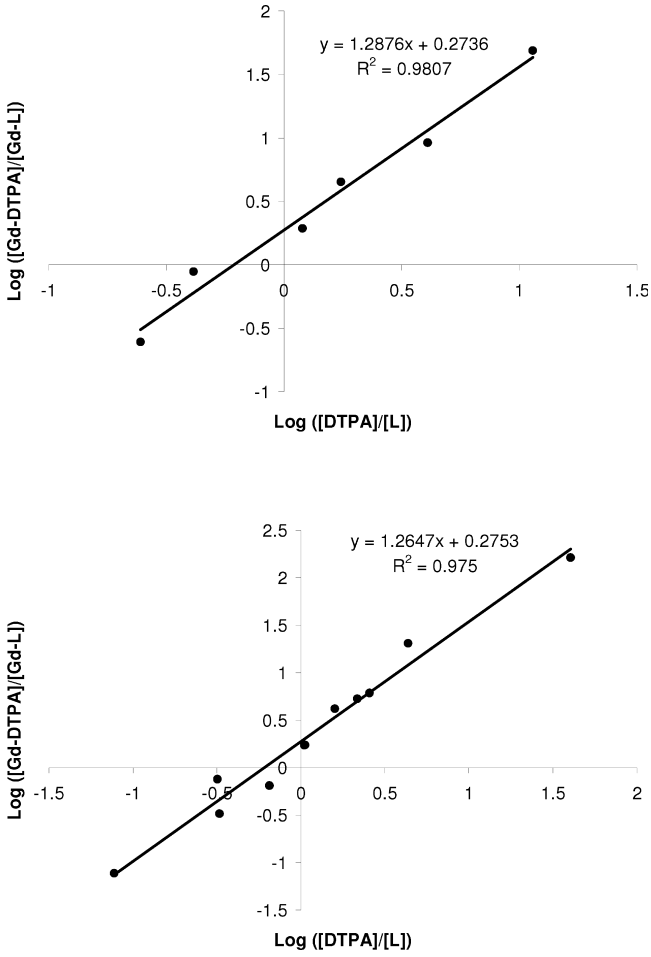

Figure 4. Competition titration log/log plots for **7** (top) and **8** (bottom) against DTPA. The *x* intercepts indicate the difference in pGd between the ligands and DTPA.

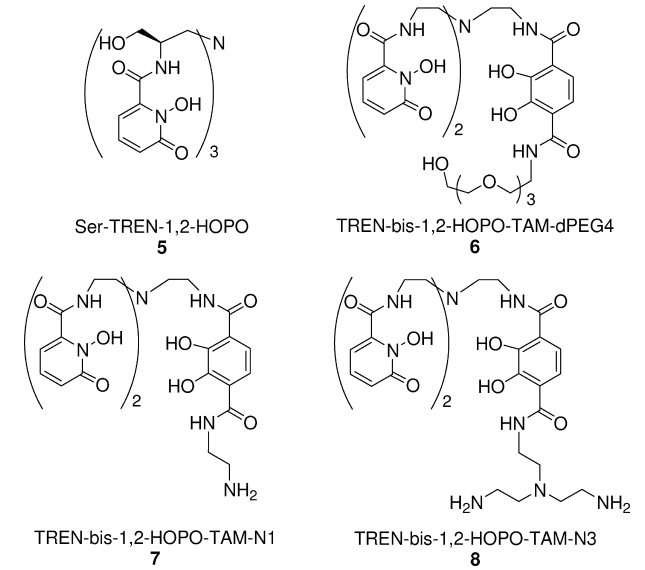

Figure 5. Chemical structures of ligands relevant to the current study.

recent communication reporting an octadentate derivative,[16] reveal the promise demonstrated by 1,2-HOPO-based ligands as MRI contrast agent precursors.

With these initial results, further study of 1,2-HOPO-based Gd(III) complexes is desirable to gain a better understanding of the parameters influencing relaxivity and to expand the scope of using such systems in practical MRI contrast agent applications. The work reported herein includes the study of a new series of both homo- and heteropodal 1,2-HOPO complexes of Gd(III). Synthesis and relaxivity studies are reported for a tris-1,2-HOPO complex that possesses a TREN ligand cap derivatized with solubilizing hydroxyl groups. Increasing the water solubility of 1,2-HOPO-based complexes is critical for the full characterization and development of such compounds as practical imaging agents. In efforts to attain even higher water solubility, a series of TREN-bis-1,2-HOPO-TAM ligands is designed and evaluated with respect to relaxivity and stability properties. While the heteropodal bis-HOPO-TAM motif has been used extensively with 3,2-HOPO-based Gd(III) chelates, the ligands reported herein represent the first such bis-HOPO-TAM compounds utilizing the 1,2-HOPO chelator. From solution thermodynamic measurements, insight is gained into the effect of a more acidic chelator on ligand protonation and Gd(III) binding for this new series of heteropodal 1,2-HOPO-based ligands. Direct measurement of the water exchange rate for one of the more soluble complexes is also achieved, representing the first such measurement for a Gd(III) complex incorporating 1,2-HOPO.

Experimental Section

Synthesis. General. Unless otherwise noted, starting materials were obtained from Aldrich Chemical Co. and used without further purification. All organic extracts were dried over $MgSO_4$, and solvents were removed with a rotary evaporator. Flash chromatography was performed on Merck Silica Gel (40−7 Mesh). Unless otherwise noted, ^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker AMX 300, 400, or DRX 500 instrument at 300, 400, or 500 MHz, and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker DRX 500 instrument at 125 MHz. The residual solvent peak was used as an internal reference. Elemental analyses were performed by the Microanalytical Laboratory at the College of Chemistry at the University of California, Berkeley, and mass spectra (LRFAB-MS = low resolution fast atom bombardment mass spectrometry; ES-MS = electrospray mass spectrometry) were performed by the Mass Spectrometry Laboratory at the College of Chemistry at U.C., Berkeley.

***N*-Boc-*O*-Bn-*R*-Ser-TREN.** The N-BOC protected derivative of **11** was synthesized according to a procedure modified from the previously reported work.[17] In this work, silica column chromatography was used with hexanes/ethyl acetate as the eluting solvent combination, with a gradient of increasing EtOAc concentration. The pure desired product was eluted with ease using Hex/EtOAc (90:10). Removal of solvents in vacuo yielded the white, waxy solid, with successful synthesis verified by a ^{1}H NMR matching the reported spectrum (2.40 g, 75.9%).

Ser-TREN-Bn-1,2-HOPO-Bn (12). A solution of 1,2-HOPO-Bn-thiaz (**10**; 0.651 g, 1.88 mmol) in CH_2Cl_2 (10 mL) was added to (*R*)-Bn-Ser$_3$-TREN (**11**; 0.289 g, 0.570 mmol) dissolved in CH_2Cl_2 (4 mL). The solution was stirred under N_2, in the dark at room temperature for 7 days, adding NEt_3 (ca. 0.2 mL) on the sixth

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day. Thin-layer chromatography indicated the formation of the mono-, bis-, and tris-Ser-TREN-substituted product, with **12** appearing as the dominant product. Solvents were removed under high vacuum conditions before redissolving the oily orange residue in CH_2Cl_2 (15 mL) and washing with 1 M KOH (2 × 15 mL). The organic fractions were washed with brine (1 × 25 mL) and dried, and the solvent was removed in vacuo. The pure product was obtained after silica column chromatography, eluting with CH_2Cl_2 and a gradient of gradually increasing MeOH concentration from 0% to 3% (0.225 g, 33.2%). Mp: 70−3 °C. ^{1}H NMR (300 MHz): δ 2.51−2.61 (m, 6H, Ser-TREN CH_2), 2.75−2.82 (m, 3H, Ser-TREN-C*H*), 3.27−3.40 (m, 6 H, Ser-TREN CH-CH_2-O), 4.32 (s, 6H, HOPO-Bn CH_2), 5.23−5.34 (m, 6H, Ser-TREN Bn CH_2), 6.23 (dd, 3H, HOPO C*H*-CO, J = 1.8, 5.1), 6.31 (dd, 3H, N*H*, J = 1.5, 5.4), 6.65−6.71 (m, 6H, HOPO C*H*-CR_3 and HOPO CH-C*H*-CH), 7.15−7.30 (m, 15H, Bn Ar*H*), 7.46−7.54 (m, 15H, Bn Ar*H*). ^{13}C NMR: δ 160.20, 158.29, 142.08, 137.71, 137.44, 133.35, 130.18, 129.17, 128.45, 128.40, 128.34, 128.27, 127.74, 127.70, 127.64, 124.17, 105.84, 79.01, 72.90, 68.96, 55.85, 48.21, 34.39. LRFAB-MS: m/z 1188 (M + H$^+$).

Ser-TREN-1,2-HOPO (5). Ser-TREN-Bn-1,2-HOPO-Bn (**12**; 0.136 g, 0.114 mmol) was dissolved in glacial HOAc (4 mL), to which was added 48% HBr (4 mL). The solution was stirred in the dark for 45 min. After removal of the solvents under high vacuum conditions with heating to 40 °C, the residue was dissolved in MeOH and removed in vacuo (×4). The residue was redissolved in a minimal amount of MeOH and added to 300 mL of rapidly stirring Et_2O. After stirring overnight, the solution was cooled to 0 °C, and the precipitate was isolated by filtration as a fine, white powder (0.075 g, 89.7%). ^{1}H NMR (CD_3OD, 400 MHz): δ 3.57−3.90 (m, 12H, Ser-TREN CH_2), 4.50−4.65 (m, 3H, Ser-TREN C*H*), 6.72−6.93 (m, 6H, HOPO C*H*-CO, HOPO C*H*-C-N), 7.42−7.52 (m, 3H, HOPO CH-C*H*-CH). LRFAB-MS: m/z 648.3 (M + H$^+$). Anal. calcd for **5**·HBr·4.5H_2O (found): C, 40.06 (40.37); H, 5.35 (4.91); N, 12.11 (11.64).

Gd-Ser-TREN-1,2-HOPO (Gd-5). Ser-TREN-1,2-HOPO (**5**; 0.050 g, 0.0618 mmol) was dissolved in MeOH (8 mL), and the solution was purged with N_2 for 15 min. A solution of Gd(acac)$_3$·3H_2O (0.0314 g, 0.0618 mmol) in MeOH (2 mL) was added, followed by the addition of two drops of pyridine. The solution was refluxed (oil bath at 80 °C) for 6 h, under N_2. The MeOH solution, now with a white precipitate present, was added to a stirring solution of Et_2O (300 mL) and was stirred in the dark for 6 h. The precipitate was collected by filtration as a light tan solid and was dried overnight under a vacuum at 45 °C (0.0580 g, 97.0%). ES-MS(+): m/z 825.2 (M + Na$^+$). The isotopic distribution matches closely with the simulated spectrum. Anal. calcd for Gd-**5**·5.5H_2O (found): C, 33.03 (33.23); H, 4.31 (4.18); N, 9.99 (9.58).

TREN-bis-1,2-HOPO-Bn (14). To a solution of tris(2-aminoethyl)amine (0.149 mL, 1.00 mmol) and triethylamine (5 mL) in CH_2Cl_2 (300 mL) was added dropwise 1,2-HOPO-Bn-thiaz (**10**; 0.645 g, 1.862 mmol) in CH_2Cl_2 (200 mL) over a period of 3 days. The solvent was removed in vacuo and the residue redissolved in a minimal amount of CH_2Cl_2. Purification by silica gel column chromatography was performed with a gradually increasing gradient of methanol and triethylamine in CH_2Cl_2. The desired pure product eluted in a solution of 95:4:1 CH_2Cl_2/MeOH/NEt_3 as a viscous yellow oil (0.510 g, 45.5%). ^{1}H NMR (400 MHz): δ 7.49 (m, 4H, Bn Ar-*H*, HOPO Ar-*H*), 7.30 (m, 6H, Bn Ar-*H*), 7.18 (m, 2H, Bn Ar-*H*), 6.52 (d, 2H, HOPO Ar-*H*), 6.16 (d, 2H, HOPO Ar-*H*), 5.28 (s, 4H, Bn CH_2), 2.47−2.39 (m, 6H, TREN CH_2), 2.28 (m, 2H, TREN CH_2), 2.11 (m, 2H, TREN CH_2). ^{13}C NMR: δ 161.29, 158.92, 146.09, 143.90, 138.90, 133.80, 130.87, 130.59, 129.58, 128.70, 122.85, 105.28, 79.50, 63.28, 52.95, 38.08. LRFAB-MS(+) m/z 601 (M + H$^+$).

TAM-Bn$_2$-thiaz-dPEG4 (15). A solution of dPEG4 (0.250 g, 1.29 mmol) and NEt_3 (0.160 g, 1.58 mmol) in CH_2Cl_2 (300 mL) was slowly added dropwise to a rapidly stirring solution of TAM-Bn$_2$-thiaz$_2$ (**13**; 9.70 g, 16.70 mmol) in CH_2Cl_2 (400 mL). Addition was complete after 3 days, and the solvent was removed in vacuo. The residue was redissolved in a minimal amount of CH_2Cl_2 and loaded onto a silica column packed with CH_2Cl_2. A gradually increasing gradient of MeOH in CH_2Cl_2 was used, with the pure solid yellow product eluting with CH_2Cl_2/MeOH (97:3; 0.588 g, 69.6%). ^{1}H NMR (400 MHz): δ 2.91 (t, 2H, thiaz CH_2, J = 7.2), 3.45−3.65 (m, 16H, dPEG4 CH_2), 4.35 (t, 2H, thiaz CH_2, J = 7.2), 5.09 (s, 4H, Bn CH_2), 7.18 (d, 1H, TAM Ar-*H*, J = 8.0), 7.27−7.44 (m, 10H, Bn Ar-*H*), 7.80 (d, 1H, TAM Ar-*H*, J = 8.0), 8.11 (bt, 1H, CON*H*CH_2). ^{13}C NMR: δ 28.57, 39.57, 55.46, 61.49, 69.48, 70.04, 70.11, 70.32, 70.45, 72.37, 75.95, 124.24, 126.36, 127.85, 128.28, 128.50, 128.55, 128.60, 128.85, 130.81, 133.15, 135.75, 136.96, 149.31, 149.76, 164.47, 166.80, 201.23. LRFAB-MS: m/z 655 (M + H$^+$).

TREN-bis-1,2-HOPO-Bn-TAM-Bn$_2$-dPEG4 (16). To a solution of TREN-bis-1,2-HOPO-Bn (**14**) (0.326 g, 0.543 mmol) in CH_2Cl_2 (20 mL) were added a solution of TAM-Bn$_2$-thiaz-dPEG4 (**15**) (0.375 g, 0.573 mmol) in CH_2Cl_2 (2 mL) and several drops of triethylamine. The mixture was stirred overnight at room temperature under N_2 before the solvent was removed in vacuo and the crude product purified by silica gel column chromatography. A gradually increasing gradient of methanol in CH_2Cl_2 was used, with the benzyl-protected ligand, **16**, eluting as a viscous yellow oil in 94:6 CH_2Cl_2/MeOH (0.410 g, 66.5%). ^{1}H NMR (400 MHz) δ 8.40 (m, 1H, TAM Ar-*H*), 8.09 (m, 1H, TAM Ar-*H*), 7.68 (m, 1H, TAM Ar-*H*), 7.68−7.05 (m, 22H, Bn Ar-*H*, HOPO Ar-*H*), 6.64 (d, 2H, HOPO Ar-*H*), 6.14 (d, 2H, HOPO Ar-*H*), 5.32 (s, 4H, Bn CH_2), 5.00 (s, 2H, Bn CH_2), 4.66 (s, 2H, Bn CH_2), 3.65−3.42 (m, 16H, PEG CH_2), 3.19 (bs, 2H, TREN CH_2), 3.00 (bs, 2H, TREN CH_2), 2.43 (bs, 2H, TREN CH_2), 2.09 (bs, 2H, TREN CH_2). ^{13}C NMR δ 166.24, 165.77, 161.04, 158.84, 151.05, 149.69, 143.09, 138.72, 136.69, 136.46, 133.87, 130.60, 129.89, 129.33, 129.15, 128.85, 128.76, 128.62, 128.52, 128.03, 125.35, 124.73, 123.78, 105.55, 79.33, 72.62, 70.69, 70.59, 70.32, 69.85, 61.71, 55.13, 52.83, 50.78, 39.84, 37.37, 31.72, 29.83. LRFAB-MS(+) m/z 1160.6 (M + Na$^+$).

TREN-bis-1,2-HOPO-TAM-dPEG4 (6). TREN-bis-1,2-HOPO-Bn-TAM-Bn$_2$-dPEG4 (**16**; 0.410 g, 0.361 mmol) was dissolved in a 1:1 mixture of 12 M HCl/glacial acetic acid (40 mL). The mixture was stirred at room temperature overnight under N_2. The solvent was removed exhaustively in vacuo, and the residue was dissolved in a minimal amount of MeOH. Deprotected ligand **6** was precipitated in Et_2O (400 mL) over 48 h to yield a white solid, which was dried under high vacuum conditions at 45 °C (0.126 g, 45%). ^{1}H NMR (400 MHz): δ 9.09 (m, 4H, TAM amide *H*, HOPO amide *H*), 7.40 (m, 4H, TAM Ar-*H*, HOPO Ar-*H*), 6.63 (d, 2H, HOPO Ar-*H*), 6.40 (d, 2H, HOPO Ar-*H*), 3.53−3.39 (m, 28H, PEG CH_2, TREN CH_2). LRFAB-MS(+): m/z 776 (M + H$^+$). Anal. calcd for 2−12·HCl·3H_2O (found): C, 47.14 (47.38); H, 6.05 (5.79); N, 11.32 (10.99).

TREN-bis-1,2-HOPO-Bn-TAM-Bn-thiaz (17). To a solution of TAM-Bn$_2$-thiaz$_2$ (**13**; 1.74 g, 3.0 mmol) in CH_2Cl_2 (200 mL) was added TREN-bis-1,2-HOPO-Bn (**14**; 0.900 g, 1.5 mmol) in CH_2Cl_2 (300 mL) and NEt_3 (8 mL) dropwise over a period of 5 days. The solvents were removed in vacuo and the residue redissolved in a minimal amount of CH_2Cl_2. Purification by silica gel column chromatography was performed with a gradually

increasing gradient of MeOH in CH_2Cl_2. The desired compound was eluted with 97:3 CH_2Cl_2/MeOH, and the solvents were removed in vacuo to yield a bright yellow solid (0.596 g, 35.2%). ^{1}H NMR (400 MHz): δ 7.78 (t, 1H, TAM amide *H*), 7.51−7.24 (m, 25H, HOPO amide *H*, Bn Ar-*H*, HOPO Ar-*H*, TAM Ar-*H*), 7.07 (d, 1H, TAM Ar-*H*), 6.67 (d, 2H, HOPO Ar-*H*), 6.20 (d, 2H, HOPO Ar-*H*), 5.32 (s, 4H, HOPO Bn CH_2), 5.09 (s, 2H, TAM Bn CH_2), 5.03 (s, 2H, TAM Bn CH_2), 4.42 (t, 2H, thiaz CH_2), 3.15 (m, 2H, TREN CH_2), 2.99−2.95 (m, 4H, TREN CH_2, thiaz CH_2), 2.45−2.43 (m, 4H, TREN CH_2), 2.23 (m, 2H, TREN CH_2). ^{13}C NMR: δ 201.61, 166.65, 165.16, 160.92, 158.55, 150.15, 149.42, 143.13, 138.49, 137.12, 135.92, 133.89, 133.73, 130.52, 129.43, 129.28, 129.10, 129.02, 128.95, 128.79, 129.67, 128.56, 128.04, 126.22, 124.64, 123.85, 105.38, 79.35, 77.13, 76.24, 55.77, 54.33, 53.02, 38.08, 37.85, 28.89. LRFAB-MS(+): *m*/*z* 1069 (M + Li$^+$).

TREN-bis-1,2-HOPO-Bn-TAM-Bn-N1-BOC (18). To a solution of TREN-bis-1,2-HOPO-Bn-TAM-Bn-thiaz (**17**; 0.298 g, 0.281 mmol) in CH_2Cl_2 (30 mL) was added mono-BOC-protected ethylene diamine (N1; 0.045 g, 0.281 mmol) and triethylamine (∼0.3 mL). The mixture was stirred overnight at room temperature under N_2, and the solvent was removed in vacuo. The crude product was purified by silica gel chromatography and eluted in an increasing gradient of MeOH in CH_2Cl_2. The desired product was isolated as a white solid (0.299 g, 96.6%). ^{1}H NMR (400 MHz): δ 8.27 (m, 1H, TAM amide *H*), 8.21 (m, 1H, TAM amide *H*), 7.75 (bs, 2H, HOPO amide *H*), 7.47−7.14 (m, 23H, Bn Ar-*H*, HOPO Ar-*H*, TAM Ar-*H*), 6.95 (m, 3H, TAM Ar-*H*, HOPO Ar-*H*), 6.62 (d, 2H, HOPO Ar-*H*), 6.26 (m, 1H, BOC amide *H*), 6.10 (d, 2H, HOPO Ar-*H*), 5.33 (s, 4H, HOPO Bn CH_2), 4.87 (s, 2H, TAM Bn CH_2), 4.45 (s, 2H, TAM Bn CH_2), 3.53 (bs, 2H, ethylenediamine CH_2), 3.39 (m, 2H, ethylenediamine CH_2), 3.21 (bs, 4H, TREN CH_2), 3.03 (bs, 2H, TREN CH_2), 2.43 (bs, 4H, TREN CH_2), 2.02 (bs, 2H, TREN CH_2), 1.28 (s, 9H, BOC CH_3). ^{13}C NMR: δ 166.46, 165.77, 161.01, 158.89, 156.35, 152.99, 151.20, 149.47, 143.04, 138.85, 138.30, 136.60, 135.49, 133.85, 130.57, 129.25, 128.77, 128.61, 128.54, 127.51, 126.04, 125.23, 124.25, 123.73, 105.61, 79.26, 79.00, 77.43, 76.40, 67.16, 55.58, 52.80, 50.71, 40.45, 40.29, 38.80, 37.13, 28.44. ES-MS(+): *m*/*z* 1125.5 (M + Na$^+$).

TREN-bis-1,2-HOPO-Bn-TAM-Bn-N3-diBOC (19). To a solution of TREN-bis-1,2-HOPO-Bn-TAM-Bn-thiaz (**17**; 0.298 g, 0.281 mmol) in CH_2Cl_2 (30 mL) was added TREN-diBOC (N3; 0.0974 g, 0.281 mmol) and NEt_3 (∼0.3 mL). The mixture was stirred overnight at room temperature under N_2, and the solvent was removed in vacuo. The crude product was purified by silica gel chromatography and eluted in an increasing gradient of MeOH in CH_2Cl_2. A white solid was obtained following removal of the solvent in vacuo (0.260 g, 70.9%). ^{1}H NMR (400 MHz): δ 8.42 (m, 1H, TAM amide *H*), 8.32 (m, 1H, TAM amide *H*), 7.87 (bs, 2H, HOPO amide *H*), 7.48−7.15 (m, 21H, Bn Ar-*H*, HOPO Ar-*H*, TAM Ar-*H*), 6.98 (m, 2H, HOPO Ar-*H*), 6.87 (d, 1H, TAM Ar-*H*), 6.73 (d, 2H, HOPO Ar-*H*), 6.62 (d, 1H, TAM Ar-*H*), 6.16−6.12 (m, 4H, HOPO Ar-*H*, BOC amide *H*), 5.34 (s, 4H, HOPO Bn CH_2), 4.93 (s, 2H, TAM Bn CH_2), 4.45 (s, 2H, TAM Bn CH_2), 3.56 (bs, 2H, TREN CH_2), 3.24 (bs, 4H, TREN CH_2), 3.11−3.05 (m, 6H, TREN CH_2), 2.66 (bs, 2H, TREN CH_2), 2.54 (bs, 4H, TREN CH_2), 2.45 (bs, 4H, TREN CH_2), 2.06 (bs, 2H, TREN CH_2), 1.32 (s, 18H, BOC CH_3). ^{13}C NMR: δ 166.98, 166.03, 160.97, 159.32, 156.42, 151.37, 149.38, 143.24, 138.81, 137.03, 136.72, 133.94, 130.46, 129.21, 128.82, 128.76, 128.56, 128.46, 128.35, 127.49, 125.07, 123.97, 105.79, 79.47, 79.06, 76.71, 76.31, 55.67, 55.08, 53.01, 39.25, 39.05, 38.33, 37.23, 28.54. ES-MS(+): *m*/*z* 1311.6 (M + Li$^+$).

TREN-bis-1,2-HOPO-TAM-N1 (7). TREN-bis-1,2-HOPO-Bn-TAM-Bn$_2$-N1-BOC (**18**; 0.299 g, 0.271 mmol) was dissolved in 1:1 12 M HCl/glacial acetic acid (20 mL) and stirred for 5 days at room temperature. The solvent was removed exhaustively in vacuo. The product was suspended in a minimum amount of MeOH and precipitated with Et_2O (300 mL). The resulting white solid was isolated by filtration and dried in vacuo (0.120 g, 68.9%). ^{1}H NMR (CD_3OD, 400 MHz): δ 7.30 (t, 2H, HOPO Ar-*H*), 7.05 (d, 1H, TAM Ar-*H*), 6.98 (d, 1H, TAM Ar-*H*), 6.69 (m, 4H, HOPO Ar-*H*), 3.88 (bs, 6H, TREN CH_2), 3.61 (m, 8H, TREN CH_2, ethylenediamine CH_2), 3.10 (m, 2H, ethylenediamine CH_2). ^{13}C NMR (CD_3OD): δ 171.65, 171.45, 163.76, 159.92, 150.89, 149.97, 140.00, 138.01, 121.20, 119.43, 118.89, 118.60, 117.91, 110.79, 56.15, 55.50, 41.06, 38.52, 36.57, 36.45. LRFAB-MS(+): *m*/*z* 643 (M + H$^+$). Anal. calcd for **7**·3HCl·2H_2O·0.5MeOH (found): C, 42.53 (42.76); H, 5.29 (5.20); N, 13.93 (13.28).

TREN-bis-1,2-HOPO-TAM-N3 (8). TREN-bis-1,2-HOPO-Bn-TAM-Bn$_2$-N3-diBOC (**19**; 0.260 g, 0.199 mmol) was dissolved in 1:1 12 M HCl/glacial acetic acid (20 mL) and stirred for 2 days at room temperature. The solvent was removed exhaustively in vacuo. The product was suspended in a minimum amount of methanol and precipitated with Et_2O (300 mL). The resulting white solid was isolated by filtration and dried in vacuo (0.136 g, 94.1%). ^{1}H NMR (D_2O): δ 7.33 (t, 2H, HOPO Ar-*H*), 7.01 (dd, 2H, TAM Ar-*H*), 6.76 (d, 2H, HOPO Ar-*H*), 6.15 (d, 2H, HOPO Ar-*H*), 3.90 (m, 6H, TREN CH_2), 3.72 (m, 6H, TREN CH_2), 3.54 (t, 2H, TREN CH_2), 3.13 (t, 4H, TREN CH_2), 2.92 (t, 4H, CH_2), 2.86 (t, 2H, TREN CH_2). Anal. calcd for **8**·4HCl·2.5H_2O·MeOH (found): C, 41.65 (41.85); H, 6.04 (5.86); N, 14.72 (14.58).

pK_a and Complex Formation Constant Determination by Potentiometric and Spectrophotometric Titration. Details of the experimental procedures and equipment used to determine ligand pK_a's and complex formation constants for TREN-bis-1,2-HOPO-TAM-dPEG4 (**6**) can be found elsewhere.[12] All experiments were performed at 25 °C in 0.1 M KCl. Each determination resulted from at least three independent experiments, each consisting of two titrations (first with acid, then reverse with base). Equilibration times used were 210 s for potentiometry, and 300 s for spectrophotometry. Ligand concentrations were ca. 1 mM and ca. 60 μM for potentiometry and spectrophotometry, respectively. A pH range of 2.5−11 was used, and the data were processed by the Hyperquad[18] and pHAB[19] suite of computer programs. The complex formation constants of Gd-**6** were determined by nonlinear least-squares fits of spectrophotometric data using pHAB over the wavelength range of 280−430 nm. The β_{110} and β_{111} values derived from the refinement were 21.68 and 27.05, respectively, and the pGd was calculated in HySS[20] using these values and the protonation constants from potentiometry.

Competition Batch Titrations for pGd Determination. The general procedure used to determine the pGd values of **5**, **7**, and **8** was described previously.[7,21] Different volumes of a standardized DTPA stock solution were added to solutions of constant ligand, metal, and electrolyte concentrations. In the current work, the pH of all solutions was kept constant at 7.4 with a HEPES buffer instead of adjusting the pH to 6.0 as was done in past studies,[21] and the solutions were diluted to identical volumes. After stirring the solutions for 24 h to ensure that thermodynamic equilibrium was reached, the pH was again checked just before analyzing the samples spectrophotometrically. The concentrations of each ligand relative to DTPA used in the final data analysis ranged from 1:0.1

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to 1:10 (L/DTPA). Concentrations of free and complexed ligands in each solution were determined from the absorption spectra and spectra of free and fully complexed ligands at identical pH's and concentrations used as references for the analysis. These concentrations were used for the log/log plots (Figures 3 and 4) to give the difference in pGd between the competing DTPA and ligand of interest.

Relaxivity Measurements. ^{1}H NMR. The water proton longitudinal relaxation rates of 1 mM aqueous solutions of complexes Gd-**5**, Gd-**6**, Gd-**7**, and Gd-**8** were measured by using a Stelar Spinmaster spectrometer (Mede, Italy) operating at 0.5 T and 25 °C. Solutions were prepared in situ by titrating a solution of the ligand of a known concentration with the appropriate amount of standardized Gd(III) stock solution and adjusting the pH to 7.4. For a measurement of relaxation rates, the standard inversion−recovery method was employed (16 experiments, four scans) with a typical 90° pulse width of 3.5 ms, and the reproducibility of the T_1 data was ±0.5%. The temperature was controlled with a Stellar VTC-91 air-flow heater equipped with a copper-constantan thermocouple (uncertainty of ±0.1 °C). The proton $1/T_1$ NMRD profiles were measured on a fast field-cycling Stelar Spinmaster FFC relaxometer over a continuum of magnetic field strengths from 0.00024 to 0.5 T (corresponding to 0.01−20 MHz proton Larmor frequencies). The relaxometer operates under computer control with an absolute uncertainty in $1/T_1$ of ±1%. Additional data points at 20−70 MHz were obtained on a Stelar Relaxometer.

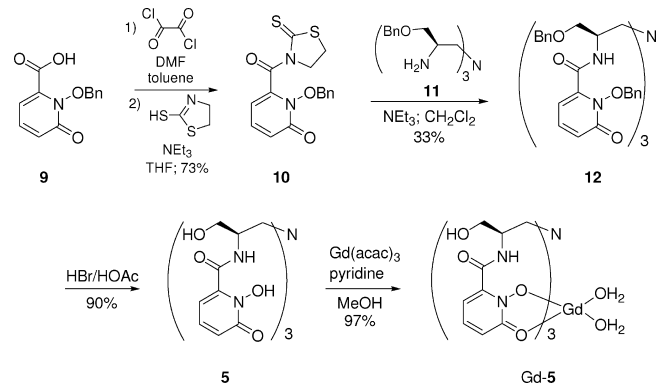
^{17}O NMR. Variable-temperature ^{17}O NMR measurements were recorded on a JEOL EX-90 (2.1 T, 12 MHz) spectrometer equipped with a 5 mm probe and standard temperature control units. An aqueous solution of Gd-**8** (8.3 mM, pH = 7.4) containing 2.0% of the ^{17}O isotope (Cambridge Isotope) and a D_2O external lock were used. The observed transverse relaxation rates were calculated from the signal width at half height.

Results and Discussion

Ligand Design and Synthesis. The first 1,2-HOPO-based Gd(III) complex prepared in the current study was Gd-Ser-TREN-1,2-HOPO (Gd-**5**; Figure 5). This complex is analogous to the previously reported Gd-Ser-TREN-1-Me-3,2-HOPO,[17,22] which utilized a tris-hydroxyl derivative of TREN as a ligand cap for enhanced solubility in water. Use of this so-called "Serine-TREN" or Ser-TREN cap gave a water solubility of about 15 mM (25 °C, pH 7) for the tris-3,2-HOPO complex, representing an increase in solubility of more than 100-fold relative to the parent Gd-TREN-1-Me-3,2-HOPO (**1**). The resultant higher solubility allowed for a much more detailed relaxometric investigation, including acquisition of the water exchange rate via variable-temperature ^{17}O NMR experiments to yield a τ_M value of 16 ns.[22] Inspired by this study, the Ser-TREN cap was chosen in the synthesis of the tris-1,2-HOPO analog Gd-**5**.

The synthesis of Gd-Ser-TREN-1,2-HOPO (Gd-**5**) was carried out according to Scheme 1. After synthesizing the benzyl-protected 1,2-HOPO acid (**9**) according to literature procedure,[23,24] the acid precursor was activated with 2-mercaptothiazoline for subsequent coupling with the primary

Scheme 1. Synthesis of Gd-Ser-TREN-1,2-HOPO (Gd-**5**)



amines of Ser-TREN (**11**). After an optimized chromatographic procedure to obtain the previously reported N-Boc-O-Bn-R-Ser-TREN[17] (see Experimental Section) and BOC deprotection to give **11**, coupling to the Ser-TREN backbone was accomplished by stirring in CH_2Cl_2 for 1 week with NEt_3. Formation of the mono-, bis-, and tris-substituted TREN backbone was observed, with **12** appearing as the dominant product by day seven. After silica column chromatography to afford the protected ligand, full benzyl deprotection was then pursued.

In previous work by Hajela et al., benzyl deprotection of the Ser-TREN-3,2-HOPO ligand was accomplished using HBr/HOAc at room temperature.[17] Such strongly acidic conditions were required to ensure complete deprotection of the Ser-TREN cap benzyl groups. This approach was cautiously applied to the 1,2-HOPO compound in this work, as the $N-O_{hydroxy}$ bond of 1,2-HOPO is relatively weak and susceptible to cleavage by HBr. It is also noted that standard conditions such as BBr_3 and H_2/Pd−C caused decomposition in past 1,2-HOPO deprotection attempts.[25] Following trials employing various reaction times and temperatures, the pure ligand **5** was obtained by stirring in HBr/HOAc at room temperature for only 45 min to allow just enough time for complete deprotection of all six benzyl groups, but not enough for partial decomposition of the 1,2-HOPO chelator. Following deprotection, complexation with Gd(III) using the acetylacetonate salt of the metal afforded the desired Gd-Ser-TREN-1,2-HOPO complex (Gd-**5**).

Another approach to generate 1,2-HOPO complexes of high water solubility involves the preparation of bis-HOPO-TAM analogs for the attachment of solubilizing groups via the available amide functionality of the terephthalamide (TAM). The ligands shown in Figure 5 represent the first series of heteropodal 1,2-HOPO-TAM ligands to be studied as Gd(III) chelators and should afford complexes with solubilities high enough for full relaxometric characterization, including variable-temperature ^{17}O NMR experiments required for direct water exchange measurement. Synthesis of these ligands was accomplished according to Schemes 2 and

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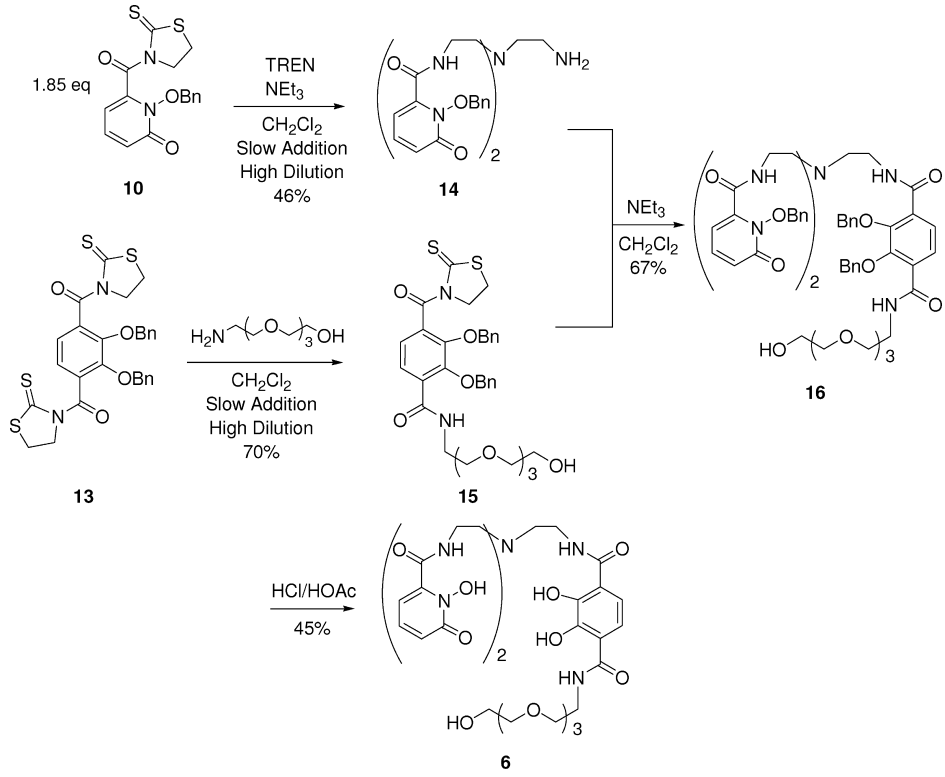
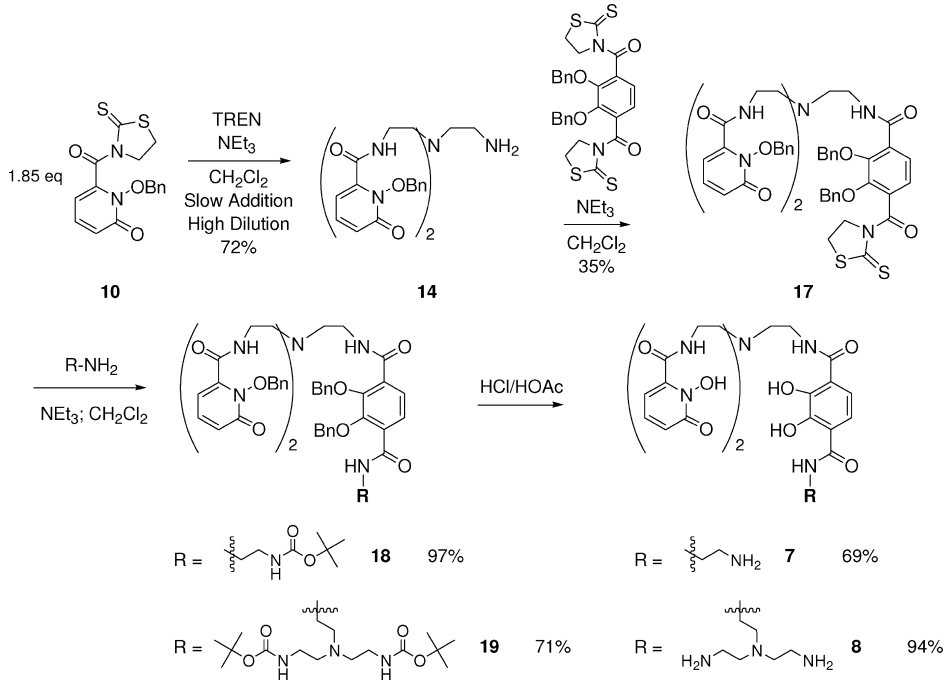
Scheme 2. Synthesis of TREN-bis-1,2-HOPO-TAM-dPEG4 (**6**)



Scheme 3. Synthesis of TREN-bis-1,2-HOPO-TAM-N1 (**7**) and TREN-bis-1,2-HOPO-TAM-N3 (**8**)



3. Common to the syntheses of compounds **6**, **7**, and **8** is the generation of the TREN-bis-1,2-HOPO precursor **14**, prepared under high-dilution, slow-addition conditions for asymmetric substitution of TREN at only two of the three primary amines. Following silica column chromatography, **14** was coupled with the appropriate monosubstituted TAM podand, in the case of the polyethylene glycol (dPEG4) derivative **16** (Scheme 2), or the dithiazolide-activated TAM, in the cases of **18** and **19** (Scheme 3). The latter method generated the useful TREN-bis-1,2-HOPO-TAM-thiaz compound (**17**), which can be stored for long periods of time yet is highly reactive toward primary amines for amide coupling with a variety of substituents. For the ethylenediamine (N1) and TREN (N3) substituted ligands, the amines of each substituent were first asymmetrically protected with BOC-anhydride, as was done previously[26] to give the mono- and disubstituted amines used in subsequent coupling to TREN-bis-1,2-HOPO-TAM-thiaz (**17**). Full deprotection of

all three of the heteropodal ligands was achieved under acidic conditions of HCl/HOAc.

Solution Thermodynamics. One practical concern related to the use of 1,2-HOPO ligands as Gd chelators for MRI applications is complex stability. The 1,2-HOPO moiety is much more acidic than the 3,2-HOPO chelator (pK_a of 5.8 versus 8.7[27]), and past work has determined an optimal ligand basicity for maximizing the thermodynamic stability of Gd(III) complexes.[21] This might suggest that, due to the significant decrease in basicity, 1,2-HOPO ligands may produce less-stable Gd(III) complexes. Spectrophotometric titration studies were thus performed to determine the pM value for Ser-TREN-1,2-HOPO (**5**) with Gd. Analogous to pH, pM is defined as the −log of the concentration of free metal in solution (eq 1) at some specified set of standard conditions (typically $[M]_T = 1\ \mu M$, $[L]_T = 10\ \mu M$, pH 7.4, 25 °C, and 0.1 M KCl).

$$pM = -\log[M^{n+}]_{free} \quad (1)$$

The method chosen to determine this conditional stability constant was a competition batch titration with DTPA as the competing ligand.[7,21] In this experiment, the concentrations of **5** and Gd(III) as well as the pH were kept constant while the concentration of DTPA was progressively increased. Figure 3a shows the absorption spectra obtained for the free ligand **5** and the 1:1 Gd-**5** complex, with the intermediate spectra corresponding to the complex in the presence of varying amounts of DTPA. From the absorption data, the resulting concentrations of the free and complexed ligands were determined, and a plot of log([Gd-DTPA]/[GdL]) versus log([DTPA]/[L]) was prepared (Figure 3b), giving the difference in pM between **5** and DTPA (log([DTPA]/[L] when log([Gd-DTPA]/[GdL] = 0, or when the concentration of DTPA generates an equal partition of Gd between **5** and DTPA). Using the known pGd of 19.1 for DTPA,[28] the pGd of **5** was calculated to be 18.5(1). This value is slightly lower than that of DTPA, but still significantly higher than that of DTPA-BMA (pGd = 15.8[2]), another ligand used clinically. Also noteworthy is the slight decrease in stability upon replacing the TREN cap with Ser-TREN. The pGd for TREN-1,2-HOPO was recently determined to be 19.3(2),[15] indicating a 0.8 log unit decrease in stability for **5** relative to the parent TREN-capped compound. The same effect is observed in the pGd decrease of Gd-Ser-TREN-3,2-HOPO relative to Gd-TREN-3,2-HOPO and may be due to the conformational constraints imposed upon substitution of the TREN cap or an increase in ligand acidity for the Ser-TREN capped compounds.[17] While a decrease in stability as compared with other TREN-capped 3,2-HOPO-based ligands is observed, the change is relatively small (pGd = 19.2 for TREN-1-Me-3,2-HOPO, for example) and should not prevent the use of such 1,2-HOPO ligands in future contrast agent applications.

Table 1. pKa's of TREN-bis-1,2-HOPO-TAM-dPEG4 (**6**) Compared to Those Previously Reported for TREN-bis-3,2-HOPO-TAM-Me (**2**)[a]

	6	**2**[21]
log K_1	10.09(2)	11.42(7)
log K_2	7.15(3)	8.14(3)
log K_3	5.87(3)	7.05(1)
log K_4	4.29(2)	5.75(1)
log K_5	3.26(2)	4.98(6)

[a] All constants determined at 25 °C in 0.1 M KCl.

Encouraged by the promising stability results obtained for Ser-TREN-1,2-HOPO (**5**), we carried out a more detailed solution thermodynamics study for the heteropodal bis-1,2-HOPO-TAM ligands. TREN-bis-1,2-HOPO-TAM-dPEG4 (**6**) was chosen as the best ligand for pK_a measurements via potentiometric titrations due to the lack of extra amine protonation steps, which would likely complicate data interpretation for ligands such as **7** and **8**. Three independent experiments were performed, with each experiment consisting of one "forward" titration (acidic to basic pH) and one "reverse" titration (basic to acidic pH). Each run was found to be reversible, giving similar results when refined separately using the Hyperquad program.[18] Refinement of all runs together gave the pK_a's shown in Table 1. The pK_a values for TREN-bis-3,2-HOPO-TAM-Me (**2**)[21] are also given for comparison, and it is seen that incorporation of the more acidic 1,2-HOPO chelator shifts each pK_a 1 to 1.5 log units lower than what is found for the 3,2-HOPO derivative. This results in an overall $\sum$ log K_a value[29] for **6** of 27.40 versus a value of 32.36 for TREN-bis-3,2-HOPO-TAM-Me.

The pGd values of the three heteropodal ligands **6**, **7**, and **8** were also determined to assess thermodynamic stability with Gd(III). When the the pK_a values of the PEG-substituted ligand **6** were combined with the formation constants obtained via variable pH spectrophotometric titrations, the HySS program[20] was used to calculate a pGd value of 19.7 for **6**. To determine this parameter for the amine-substituted 1,2-HOPO-TAM ligands, competition batch titrations versus DTPA were employed. The setup was similar to that used for the pGd determination of Ser-TREN-1,2-HOPO (**5**) and resulted in the log/log plots shown in Figure 4. Using the known pGd of DTPA, pGd's of 18.9 were measured for both amine-substituted ligands **7** and **8**. These values compare well with those obtained for commercial contrast agents (Table 2) and predict low toxicity for in vivo applications.

Relaxivity Measurements. Having confirmed adequate stability, relaxometric analysis of Gd-**5** was pursued, and a relaxivity value of 10.5 $mM^{-1}\ s^{-1}$ (20 MHz, 25 °C) was obtained. This value is slightly higher than those obtained for the originally reported TREN-capped 1,2-HOPO-based Gd(III) complexes, as shown in Table 3, and is more than twice the value of currently used clinical agents. The magnetic field dependence of the relaxivity was also recorded and fit to theory using the Solomon−Bloembergen−Morgan (innersphere contribution) and Freed (outer sphere contribu-

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Table 2. pGd Values of Selected HOPO-Based and Commercial Aminocarboxylate Complexes

complex	pGd[a]
1	19.2[b]
3	19.3[c]
Gd-**5**	18.5
Gd-**6**	19.7
Gd-**7**	18.9
Gd-**8**	18.9
Gd-DTPA	19.1[d]
Gd-DTPA-BMA	15.8[e]

[a] At pH 7.4, 25 °C. [b] From ref 21. [c] From ref 15. [d] From ref 28. [e] From ref 2.

tion) equations[2,30,31] to generate the nuclear magnetic relaxation dispersion (NMRD) profile shown in Figure 6, with best-fit parameters given in Table 3. For comparison, NMRD profiles of **3** and **4** as well as Gd-TREN-bis-1-Me-3,2-HOPO-TAM (**2**) are also shown. The curve for Gd-**5** possesses the same general features of the HOPO family of complexes shown in Figure 6, with the highest relaxivities appearing in the low-field range, a small dip at 10 MHz, and a peak in the high-field region of the profile (~100 MHz). The curve for the new tris-1,2-HOPO complex Gd-**5** supports the same trend that was noted in the past work describing 1,2-HOPO Gd complexes, where the tris-1,2-HOPO had higher r_1 values, most notably throughout the low-frequency range, followed by bis-(1-Me-3,2-HOPO)-1,2-HOPO and finally bis-(1-Me-3,2-HOPO)-TAM.[14] This behavior appears to be the consequence of rather long electronic relaxation times associated with complexes of the 1,2-HOPO family, as clearly shown by the trend of Δ^2 values in Table 3. The slight increase in relaxivity exhibited by Gd-**5** relative to other similar complexes (most r_{1p} values are ~7−9 mM^{-1} s^{-1} for HOPO $q = 2$ complexes[5]) may also arise from a small second-sphere relaxation contribution from water molecules hydrogen-bonded to the exposed hydroxyl groups of the Ser-TREN cap. However, the existence of a coordination equilibrium that includes a minor component of the nine-coordinate, $q = 3$ complex may also explain the increase and will be discussed further below.

Relaxivities of the Gd(III) complexes of TREN-bis-1,2-HOPO-TAM-dPEG4 (Gd-**6**), TREN-bis-1,2-HOPO-TAM-N1 (Gd-**7**), and TREN-bis-1,2-HOPO-TAM-N3 (Gd-**8**) were determined to be 7.8, 8.8, and 9.3 mM^{-1} s^{-1} (20 MHz, 25 °C, pH = 7.4), respectively. The relaxivity values of Gd-**7** and Gd-**8** are very similar to those reported for related 1,2-HOPO complexes,[14] and the value for Gd-**6** is slightly lower, possibly due to partial water displacement by the PEG chain or to a slightly different value for the $Gd-O_{water}$ bond distance. NMRD profiles were measured (Figure 7), and the best-fit parameters, given in Table 4, reveal values that are typical for such small-molecule, HOPO-based complexes.[7,14] Noteworthy are the parameters describing electronic relaxation which suggest again slightly longer T_{1e} times than what are observed for the corresponding 3,2-HOPO derivatives. This increase in T_{1e} is consistent with a trend that can be observed for 1,2-HOPO complexes noted above, where an increase of the electronic relaxation time is seen with an increasing number of 1,2-HOPO chelators in the complex. Also, the relaxivity value of 8.8 mM^{-1} s^{-1} for Gd-**7** indicates that, unlike what was previously observed for the bis-3,2-HOPO-TAM-N1 derivative,[26] an increase in hydration number is not apparent for the bis-1,2-HOPO-TAM-N1 complex. The relatively low relaxivity of Gd-**7** suggests that the hydrogen-bonding of an additional water molecule described for the 3,2-HOPO analog is not present in Gd-**7**.

Water Coordination and Exchange Rate Analysis. The ability of the 1,2-HOPO chelator to sensitize europium luminescence has made possible the direct measurement of the number of coordinated water molecules (q) in the Eu(III) analog of the corresponding Gd(III) HOPO chelate.[6,15] While the TAM chelator appears to quench metal-centered luminescence in the bis-1,2-HOPO-TAM complexes, the sensitizing properties of 1,2-HOPO can be utilized in directly assessing q for Eu-Ser-TREN-1,2-HOPO (Eu-**5**). An aqueous solution of Ser-TREN-1,2-HOPO (**5**) was prepared and titrated with Eu(III) to form the complex in situ. The luminescent lifetimes of Eu-**5** at pH 6 in water and in D_2O were determined (see the Supporting Information), and using the updated Horrocks equation,[32] q was calculated as 2.2 ± 0.5. This measurement confirms two coordinated water molecules, as expected by the relaxivity value of 10.5 mM^{-1} s^{-1} and suggested by the NMRD profile fitting (Figure 6). The noninteger value for the hydration state may indicate a minor component of the nine-coordinate, $q = 3$ species in coordination equilibrium with the eight-coordinate $q = 2$ species. It is known that, for Eu(III) coordination environments possessing different innersphere hydration numbers in fast exchange, the value determined for q will typically be nonintegral and represent a concentration-weighted average of the values for each environment.[32] This may explain the slight increase in relaxivity of Gd-Ser-TREN-1,2-HOPO (Gd-**5**) relative to the parent Gd-TREN-1,2-HOPO (**3**).

While it was hoped that Ser-TREN would provide the solubility required for direct water exchange measurement of this 1,2-HOPO compound as it did for the 3,2-HOPO derivative, this is not the case. The hydrophobic 1,2-HOPO chelator appears to dictate the solubility properties in water, and the enhanced solubility imparted by the cap is insufficient as the solubility of Gd-**5** is less than 1 mM at pH 7.4. Consequently, only an estimate of τ_M can be obtained from NMRD profile analysis. For Gd-**5**, a good fit was obtained with an assumed τ_M value of 10 ns, comparable with the upper limit value of ca. 50 ns reported for the other 1,2-HOPO complexes.[14]

Gd-TREN-bis-1,2-HOPO-TAM-N3 (Gd-**8**), on the other hand, had sufficient water solubility to allow direct measurement of the water exchange rate. The temperature dependence of the paramagnetic contribution to the water ^{17}O NMR transverse relaxation rate, R_{2p}, was monitored, and the plot of the data is shown in Figure 8. Over the entire temperature range investigated (275−340 K), R_{2p} increases with decreasing T, thus

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Table 3. Relaxivities of Relevant 1,2-HOPO Complexes and NMRD Best-Fit Parameters[a]

complex	r_{1p} ($mM^{-1}s^{-1}$)[b]	q	τ_R (ps)	r_{Gd-H} (Å)	τ_V (ps)	Δ^2 (s^{-2})
Gd-**5**	10.5	2	119	3.00	25.2	4.9×10^{19}
TREN-1,2-HOPO (**3**)[14]	9.5	2	104	3.00	23.4	6.6×10^{19}
TREN-bis-1-Me-3,2-HOPO-1,2-HOPO (**4**)[14]	9.3	2	116	3.00	20.6	7.1×10^{19}

[a] Experimental conditions: 25 °C, pH 7.4. [b] Relaxivities measured at 20 MHz.

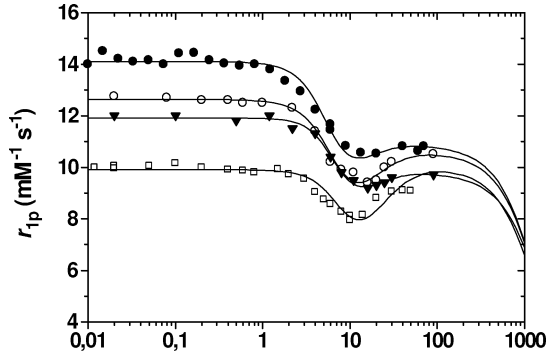

Figure 6. $1/T_1$ NMRD profiles of Gd-**5** (filled circles) and of other relevant HOPO-Gd complexes (from ref 14): Gd-TREN-1,2-HOPO (**3**, open circles), Gd-TREN-bis-1-Me-3,2-HOPO-1,2-HOPO (**4**, filled triangles), and Gd-TREN-bis-1-Me-3,2-HOPO-TAM (**2**, open squares).

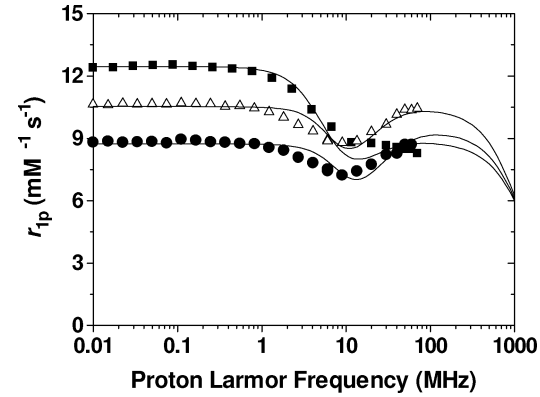

Figure 7. $1/T_1$ NMRD profiles for Gd-TREN-bis-1,2-HOPO-TAM-dPEG4 (Gd-**6**; filled circles), Gd-TREN-bis-1,2-HOPO-TAM-N1 (Gd-**7**; filled squares), and Gd-TREN-bis-1,2-HOPO-TAM-N3 (Gd-**8**; open triangles).

Table 4. NMRD Best-Fit Parameters for Heteropodal TREN-bis-1,2-HOPO-TAM Complexes[a]

parameter	Gd-**6**	Gd-**7**	Gd-**8**
Δ^2 (s^{-2})	1.3×10^{20}	6.8×10^{19}	7.9×10^{19}
τ_V (ps)	16	17	21
τ_R (ps)	129	119	148
τ_M (ns)	2[c]	2[c]	2 ± 0.2[b]
r_{Gd-H} (Å)[b]	3.1	3.1	3.1
q[c]	2	2	2

[a] Experimental conditions: 25 °C, pH 7.4. [b] From VT ^{17}O NMR data. [c] Fixed prior to fitting.

indicating that the system is in the fast exchange regime. The data were fitted using the well-established Swift−Connick equations[33,34] and gave the following values for the enthalpy ($\Delta H^{\#}$) and entropy ($\Delta S^{\#}$) of activation: $\Delta H^{\#} = 21.2 \pm 1.2$ kJ mol^{-1} and $\Delta S^{\#} = -7.0 \pm 3.0$ J mol^{-1} K^{-1}. These values correspond to an exchange rate k_{ex} of $5.1(\pm 0.4) \times 10^8$ s^{-1} ($\tau_M = 2.0$ ns), among the fastest rates directly measured for a HOPO complex. This result indicates that 1,2-HOPO-based ligands are promising candidates for future MRI applications where higher field strengths requiring fast water exchange are used.

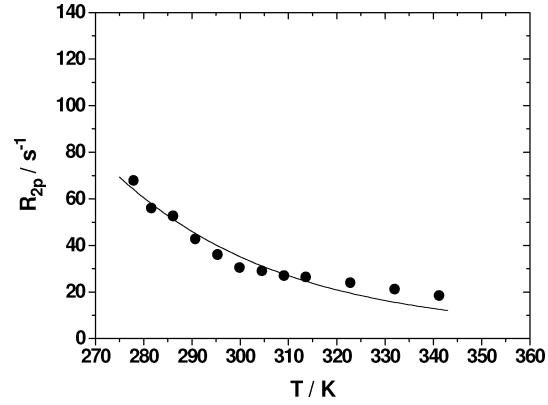

Figure 8. Temperature dependence of the paramagnetic contribution to the water ^{17}O NMR (2.1 T) transverse relaxation rate for a 8.3 mM aqueous solution of Gd-TREN-bis-1,2-HOPO-TAM-N3 (Gd-**8**). Solid curve fitted with the values of Table 4 for Δ^2 and τ_V, $E_V = 1.0$ kJ mol^{-1}, $A/\hbar = -3.50$ (± 0.2) $\times 10^6$ rad/sec, $\Delta H^{\#} = 21.2 \pm 1.2$ kJ mol^{-1}, and $\Delta S^{\#} = -7.0 \pm 3.0$ J mol^{-1} K^{-1}.

Conclusion

A new series of 1,2-HOPO-based complexes of Gd(III) has been prepared and evaluated with regard to their potential use as MRI contrast agents. The parent homopodal Ser-TREN-1,2-HOPO complex (Gd-**5**) exhibits sufficient stability and relaxivity properties for practical use, inspiring the synthesis of three heteropodal analogs, each possessing a terephthalamide chelator with an extra amide functionality for the attachment of solubilizing substituents. These compounds are the first bis-1,2-HOPO-TAM ligands to be evaluated as Gd(III) chelators, and solution thermodynamic studies reveal a Gd(III) binding strength comparable with that of other HOPO systems, as well as that of currently used commercial contrast agents. The high water solubility of Gd-TREN-bis-1,2-HOPO-TAM-N3 (Gd-**8**) enables the direct measurement of the water exchange rate via variable-temperature ^{17}O NMR experiments. The determined τ_M value of 2 ns is in the optimal range for reaching high relaxivity values at high magnetic field strengths. Further conjugation of such agents to macromolecules should result in increased rotational correlation times and further relaxivity enhancement. Taken together, the results of this study provide support for the use of 1,2-HOPO-based chelates as precursors for practical, high-relaxivity MRI contrast agents for current and future clinical applications.

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Supporting Information Available: Luminescence lifetime measurement details for Eu-**5** and relaxation rate versus [Gd] plot for Gd-**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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